Original Article

Circulating annexin A2 as a biomarker in patients with pancreatic cancer

ABSTRACT

Background: Pancreatic cancer (PC) is a highly lethal malignancy. There are only few predictive or prognostic markers for PC. This study was conducted to investigate the serum levels of annexin A2 (AnxA2) in patients with PC and its relationship with tumor progression and known prognostic parameters.

Materials and Methods: Serum samples were obtained on the first admission before any treatment. Serum AnxA2 levels were determined using enzyme-linked immunosorbent assay. Age- and sex-matched healthy controls were included in the analysis. All statistical tests were carried out using two-sided test, and $P \leq 0.05$ was considered statistically significant.

Results: The median age at diagnosis was 59 years. The most common metastatic site was liver in 23 patients with metastasis ($n = 19$, 83%). At the end of the observation period, thirty-two patients (97%) were dead. Thirty-nine percent of 23 metastatic patients who received palliative chemotherapy (CTx) were CTx responsive. Median overall survival of the whole group was 41.3 ± 8.3 weeks (95% confidence interval = 25–58 weeks). The baseline serum AnxA2 levels were significantly higher in patients with PC than in the control group ($P = 0.01$). Serum AnxA2 levels were significantly higher in the patients with high erythrocyte sedimentation rate ($P = 0.04$). Conversely, serum AnxA2 concentration had no prognostic role on survival ($P = 0.18$).

Conclusions: AnxA2 is identified as a novel secretory biomarker for PC, but it has no role as a prognostic or predictive marker.

KEY WORDS: Annexin A2, diagnostic, pancreatic cancer, serum

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INTRODUCTION

Pancreatic ductal adenocarcinoma (PDA) often has a poor prognosis, even when diagnosed early. It typically spreads rapidly and is seldom detected in its early stages, which is a major reason why it is a leading cause of cancer death.[1] At present, there is no agreed screening test to aid in the identification or earlier diagnosis of pancreatic cancer (PC) for the general population. It is the fourth leading cause of cancer-related death in the United States and second only to colorectal cancer as a cause of digestive cancer-related death.[2] The commonly used term "pancreatic cancer" usually refers to a ductal adenocarcinoma of the pancreas (including its subtypes), which represents about 85% of all pancreatic neoplasms.[3]

Annexin A2 (AnxA2) is a calcium-dependent phospholipid-binding protein widely distributed in the nucleus, cytoplasm, and extracellular surface of various eukaryotic cells. It has been recognized as a pleiotropic protein affecting a wide range of molecular and cellular processes. Dysregulation and abnormal expression of AnxA2 are linked to a large number of diseases, including autoimmune and neurodegenerative disease, antiphospholipid syndrome, inflammation, diabetes mellitus, and a series of cancers.[4] They participate in tumor cell adhesion, proliferation, invasion, metastasis, and tumor neovascularization, thereby playing a crucial role in cancer growth and progression.[5,6] The first association between AnxA2 and tumorigenesis was described in hepatocellular carcinoma (HCC) in 1990, in which an abundance of AnxA2 was detected.[7] Recently, a number of studies have found the increased expression of AnxA2 levels in many types of malignancies such as breast cancer,[8] colorectal cancer,[9] lung cancer,[10] HCC,[11] gastric cancer,[12] and PC.[13] In some of these cancers, it has been associated with poorer prognosis.[14-16] The serum level of AnxA2 was also found to be changed

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in patients with HCC,[17] invasive breast cancer,[18] lung cancer,[19] and colon cancer.[20] For PC, AnxA8 expression in cancer cells might involve an epigenetic mechanism and it might play an important role in calcium fluctuation-mediated HIF-1α transcriptional activation and cell viability.[21] In literature, the expression of tenascin-C and cell surface AnxA2 increases in the progression from low-grade pancreatic intraepithelial neoplasia to PC.[22] It has been found that the presence of AnxA6 extracellular vesicles in serum was restricted to PDA patients and represents a potential biomarker for PDA grade.[23] AnxA2 is found to be associated with gemcitabine resistance, tumor recurrence, and prognosis in PDA.[24-26] In this study, we aimed to investigate the serum AnxA2 levels in PDA patients and its relationship with clinicopathologic properties.

MATERIALS AND METHODS

Patients' characteristics

The data of 33 patients with histologically confirmed diagnosis of PC were recorded from their medical charts. Pathologic confirmation of PC was obtained by surgery or fine-needle aspiration biopsy. Patients were staged according to the International Union against Cancer TNM Classification.

Chemotherapy (CTx) was given to the majority of the patients with metastatic disease ($n = 20$, 61%). Regimens of single or combination CTx were selected according to the performance status of patients and extension of disease. Drug schemes were applied as follows: combination of gemcitabine with platinum or capecitabine ($n = 6$ and $n = 3$) or gemcitabine alone ($n = 11$). Response to treatment was determined by radiologically after 2–3 cycles of CTx according to the revised RECIST criteria version 1.1 by the investigators and classified as follows: complete response, partial response (PR), stable disease (SD), or progressive disease (PD). The tumor response after 2 months of CTx was used for statistical analysis. Patients with either PR or SD were classified as responders, and patients with PD were considered nonresponders.

The possible prognostic variables were selected based on those identified in the previous studies. Serum carcinoembryonic antigen (CEA) and carbohydrate antigen 19.9 levels were determined by microparticle enzyme immunoassay (Abbott Diagnostics, Chicago, IL, USA). Serum erythrocyte sedimentation rate (ESR), lactate dehydrogenase (LDH) levels, and albumin and whole blood count assays were measured at presentation in our biochemical laboratory. Serum LDH activity was determined immediately after collection by the kinetic method on a Targa-3000 autoanalyzer (Pointe Scientific Inc., Lincoln Park, MI, USA) at 37°C. The laboratory parameters were evaluated at diagnosis within the normal ranges of our institution.

For comparison of serum levels of AnxA2, age- and sex-matched 30 healthy controls were included in the analysis. Blood samples were obtained from patients with PA at the first admission. Institutional Review Board approval was obtained from each patient prior to the commencement of the study.

Measurement of serum annexin A2 levels

The AnxA2 enzyme-linked immunosorbent assay (ELISA) uses a double-antibody sandwich ELISA to determine the level of human AnxA2 in samples. Serum samples and standards are added to the wells which are precoated with human AnxA2 monoclonal antibody. Following incubation, AnxA2 antibodies labeled with biotin and combined with streptavidin–horseradish peroxidase are added to form immune complex and allowed to incubate for 30 min. Unbound material is washed away, and then, chromogen solution is added for the conversion of the colorless solution to a blue solution, the intensity of which is proportional to the amount of AnxA2 in the sample. As the effect of the acidic stop solution, the color has become yellow. The colored reaction product is measured using an automated ELISA reader (Rayto, RT-1904C Chemistry Analyzer, Atlanta, GA, USA). The results were expressed as ng/mL.

Statistical analysis

Continuous variables were categorized using median values as cutoff point. For group comparison of categorical variables, Chi-square tests or one-way ANOVA tests were used, and for comparison of continuous variables, Mann–Whitney U-test or Kruskal–Wallis tests were accomplished. Overall survival (OS) was calculated from the date of the first admission to the clinics to disease-related death or date of the last contact with the patient or any family member. Kaplan–Meier method was used for the estimation of survival distribution, and differences in OS were assessed by the logrank statistics. All statistical tests were carried out using two-sided test, and $P \leq 0.05$ was considered statistically significant. Statistical analysis was carried out using SPPS 21.0 (SPSS Inc., Chicago, IL., USA) software.

RESULTS

In a 3-year period, 33 patients with a pathologically confirmed diagnosis of PC were enrolled. The baseline histopathological characteristics and the demographic characteristics of the patients are listed in Table 1. The median age at diagnosis was 59 years, and the range was 32–84 years; majority of the patients were men ($n = 20$, 61%). The tumor was located in the head of the pancreas in 21 (64%) patients. Thirty-nine percent of 23 metastatic patients who received palliative CTx were CTx responsive. The most common metastatic site was liver in 23 patients with metastasis ($n = 19$, 83%). Surgery was performed in 8 (24%) patients; 5 (15%) patients underwent pancreaticoduodenectomy and 3 (9%) patients had palliative surgery.

The levels of serum AnxA2 assays in patients with PA and healthy controls are shown in Table 2. The baseline serum AnxA2 levels were significantly higher in patients with PA than in the control group ($P = 0.01$) [Figure 1].

Table 3 shows the correlation between the serum levels of AnxA2 and clinicopathological factors. Serum AnxA2 levels were significantly higher in the patients with high ESR ($P = 0.04$).

The median follow-up time was 26.0 weeks (range: 1.0–184.0 weeks). At the end of the observation period, thirty-two patients (97%) were dead. Median OS of the whole group was 41.3 ± 8.3 weeks (95% confidence interval [CI] = 25–58 weeks), whereas 1-year OS rate was 24.2% (95% CI = 9.5–38.9). Old age, worse performance status, having a metastatic disease, lack of liver metastases, and the CTx unresponsiveness were found to be significant prognostic factors ($P = 0.008$, $P = 0.002$, $P = 0.008$, $P = 0.02$, and $P = 0.03$, respectively). However, serum AnxA2 levels had no significantly effect on OS ($P = 0.18$) [Table 4 and Figure 2].

Table 1: General characteristics of pancreatic cancer patients

Variables	*n*
Number of patients	33
Age (years)	
Median (range)	59 (32-84)
Gender	
Male/female	20/13
PS[a]	
0/1/2/3	4/19/5/4
Weight loss[a]	
Yes/no	26/4
Jaundice[a]	
Yes/no	9/22
Surgery type[aa]	
Whipple surgery/palliative surgery	5/3
pT size[a]	
<Small (<40 mm)/≥ large (≥40 mm)	14/14
Site of lesion	
Head/corpus tail	21/10
Response to CTx	
Yes (PR + SD)/no (PD)	9/11
Metastasis	
Yes/no	23/10
ESH	
Normal (<40/h)/high (>40/h)	11/12
WBC count[a]	
Normal (<10.000/mm^3)/high (>10.000/mm^3)	22/9
Hb[a]	
Low (<12 g/dl)/normal (>12 g/dl)	12/19
PLT count[a]	
Low (<150.000/mm^3)/normal (>150.000/mm^3)	5/26
LDH[a]	
Normal (<450 IU/l)/high (>450 IU/l)	21/8
Albumin[a]	
Low (<4 g/dl)/normal (>4 g/dl)	10/17
CEA[a]	
Normal (<5 ng/ml)/high (>5 ng/ml)	19/10
CA 19.9[a]	
Normal (<38 U/ml)/high (>38 U/ml)	7/22

[a]Patients with unknown data concerning the variables are not included in the analysis, [aa]In 10 patients with nonmetastatic. PS=Performance status, pT=Pathologic tumor, CTx=Chemotherapy, PR=Partial response, SD=Stable disease, PD=Progressive disease, ESH=Erythrocyte sedimentation rate, WBC=White blood cell, Hb=Hemoglobin, PLT=Platelet, LDH=Lactate dehydrogenase, CEA=Carcinoembryonic antigen, CA=Carbohydrate antigen

Table 2: Serum annexin A2 levels in pancreatic cancer patients and healthy controls

Marker	Median (range)	
	Patients (*n*=33)	Controls (*n*=30)
Annexin A2 (μg/mL)	80.0 (11.5-546.7)	55.5 (11.7-148.3)

DISCUSSION

Annual PC incidence rates have been increasing and it is still one of the most lethal malignancies. There is little evidence about the predictive and prognostic biomarkers in this cancer type. Annexins are a multigene family of calcium- and phospholipid-binding proteins that play important roles in calcium signaling, cell motility, differentiation, and proliferation.[4] AnxA2 is a 36-kDa protein interfering with multiple cellular processes, especially in cancer progression.[27] AnxA2 is a calcium-dependent, phospholipid-binding protein found on various cell types. It is upregulated in various tumor types and plays multiple roles in regulating cellular functions, including angiogenesis, proliferation, apoptosis, cell migration, invasion, and adhesion. AnxA2 binds with plasminogen and tissue plasminogen activator on the cell surface, which leads to the conversion of plasminogen to plasmin.[6]

In PDA, high AnxA6 IHC score was correlated with the presence of tumor budding at the invasive front of tumors ($P = 0.082$) and the presence of perineural invasion ($P \leq 0.0001$) and showed a weak correlation with reduced survival ($P = 0.2242$).[28] The increased expression of AnxA2 has been reported in PC.[29] In addition, AnxA2 has also been demonstrated to play a role in cancer cell migration and invasion in PC.[30] Overexpression and cell surface translocation of AnxA2 during PDA pathogenesis suggest that AnxA2 is a PC-specific target, and this specificity is desirable to avoid autoimmunity.[31] In literature, markers such as AnxA2 and Semaphorin3D are told to be new therapeutic entities and prognostic markers of metastatic PDA.[32]

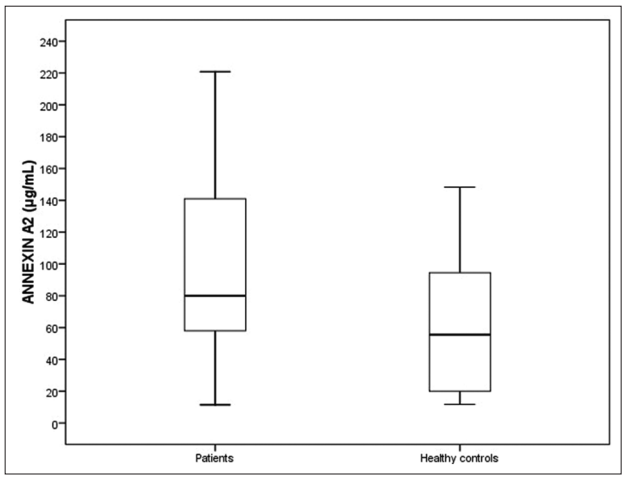

Figure 1: Serum annexin A2 levels in pancreatic cancer patients and controls ($P = 0.01$)

Table 3: Results (median and range) of comparisons between the annexin A2 marker assays and various clinical parameters

Parameters	Marker assays	
	n	Annexin A2 (μg/mL), median (range)
Age patients		
Young (<60)	18	79.5 (22.0-546.7)
Older (>60)	15	81.0 (11.5-546.7)
Gender		
Male	20	79.0 (11.5-546.7)
Female	13	85.0 (56.0-546.7)
PS		
Good (0-1)	23	79.0 (11.5-546.7)
Worse (2-4)	9	100.0 (24.0-546.7)
Weight loss		
Yes	26	79.5 (11.5-546.7)
No	4	76.5 (28.5-546.7)
Jaundice		
Yes	9	88.1 (24.0-318.0)
No	22	79.0 (11.5-546.7)
Surgery		
Yes	8	790 (11.5-546.70)
No	25	77.5 (24.0-318.0)
Localization		
Head	21	78.0 (11.5-546.7)
Corpus tail	10	113.0 (24.0-546.7)
pT size		
Small (<40 mm)	14	77.5 (11.5-546.7)
Large (≥40 mm)	14	114.6 (30.0-546.7)
Metastasis		
Yes	23	79.0 (11.5-546.7)
No	10	86.6 (28.5-546.7)
Liver metastasis		
Yes	19	80.0 (11.5-546.70)
No	4	77.5 (24.0-318.0)
ESH		
Normal	11	120.5 (22.0-546.7)
High	12	76.0 (11.5-422.8)
Hb		
Low	12	88.1 (11.5-546.7)
Normal	19	79.0 (22.0-546.7)
WBC		
High	9	85.0 (11.5-546.7)
Normal	22	79.0 (24.0-546.7)
PLT		
Low	5	58.0 (24.0-546.7)
Normal	26	80.5 (11.5-546.7)
Albumin		
Low	17	89.6 (24.0-546.7)
Normal	10	78.0 (11.5-546.7)
LDH		
High	8	80.5 (11.5-318.0)
Normal	21	85.0 (22.0-546.7)
CEA		
High	19	95.6 (11.5-546.7)
Normal	10	80.0 (22.0-546.7)
CA 19.9		
High	22	86.5 (11.5-546.7)
Normal	7	80.0 (22.0-546.7)
Response to CTx		
Yes (PR + SD)	8	74.0 (22.0-546.7)
No (PD)	7	79.0 (11.5-546.7)

PS=Performance status, pT=Pathologic tumor, CTx=Chemotherapy, PR=Partial response, SD=Stable disease, PD=Progressive disease, ESH=Erythrocyte sedimentation rate, WBC=White blood cell, Hb=Hemoglobin, PLT=Platelet, LDH=Lactate dehydrogenase, CEA=Carcinoembryonic antigen, CA=Carbohydrate antigen

Table 4: Univariate analyses of overall survival

Parameters	OS, median (±SD) (weeks)	*P*
Age patients		
Young	58.3 (13.1)	0.008*
Older	21.8 (6.6)	
Gender		
Male	49.9 (12.6)	0.21
Female	29.0 (7.5)	
PS		
Good	53.6 (10.9)	0.002*
Worse	15.6 (3.6)	
Weight loss		
Yes	36.7 (6.6)	0.34
No	74.5 (41.5)	
Jaundice		
Yes	41.6 (18.8)	0.46
No	41.9 (7.8)	
Localization		
Head	48.3 (11.8)	0.54
Corpus tail	34.4 (10.4)	
pT size		
Small	42.1 (9.4)	0.37
Large	36.4 (8.9)	
Metastasis		
Yes	26.5 (5.9)	0.008*
No	76.7 (20.3)	
Liver metastasis		
Yes	30.0 (6.8)	0.02*
No	9.5 (4.6)	
ESR		
High	34.3 (7.7)	0.24
Normal	43.9 (12.0)	
Hb		
Low	41.1 (11.5)	0.66
Normal	32.1 (7.0)	
WBC		
High	38.2 (12.2)	0.67
Normal	34.5 (7.2)	
PLT		
Low	27.5 (9.0)	0.59
Normal	37.2 (7.1)	
Albumin		
Low	30.9 (8.8)	0.79
Normal	32.8 (8.7)	
LDH		
High	24.5 (12.2)	0.06
Normal	38.3 (6.8)	
CEA		
High	30.1 (9.4)	0.66
Normal	36.8 (7.7)	
CA 19.9		
High	32.5 (6.0)	0.63
Normal	40.8 (16.0)	
Response to CTx		
Yes	48.1 (11.4)	0.03*
No	23.1 (8.9)	
Annexin A2		
<Median	52.7 (14.6)	0.18
>Median	30.0 (6.6)	

*$P \leq 0.05$. PS=Performance status, pT=Pathologic tumor, CTx=Chemotherapy, SD=Standard deviation, ESH=Erythrocyte sedimentation rate, WBC=White blood cell, Hb=Hemoglobin, PLT=Platelet, LDH=Lactate dehydrogenase, CEA=Carcinoembryonic antigen, CA=Carbohydrate antigen, OS=Overall survival

Nowadays, annexins are being investigated in PC. For example, it has been showed that the coexpression of AnxA10 and CD24 was significantly correlated with the progression of pancreatic precursor lesions toward PDAs.[33] AnxA10 is a marker which can differentiate between intrahepatic cholangiocarcinoma and hepatic metastases of PDA.[34] Specifically, Hedgehog signaling

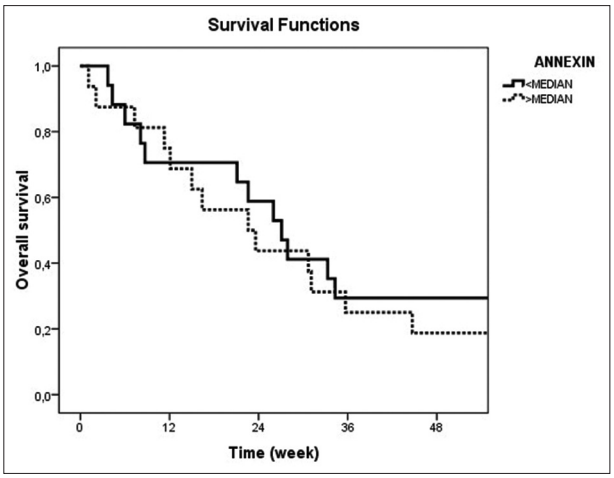

Figure 2: Overall survival curves in pancreatic cancer patients according to serum annexin A2 levels ($P = 0.18$)

from the tumor cells induces tenascin-C secretion from the stromal cells that acts back upon the tumor cells in a paracrine fashion to induce the invasion of PDA cells through its receptor AnxA2,[35] and it has been speculated that blocking the interaction between tenascin and AnxA2 has the potential to prevent liver metastasis in PDA.[35] Annexin-directed β-glucuronidase will maybe started for the targeted treatment of solid tumors.[36]

CONCLUSION

In this study, we found that AnxA2 is a potential diagnostic marker for PC. It was not prognostic or predictive. A greater understanding of the mechanisms of AnxA2 in PC and other cancers could potentially lead to the development of novel therapeutics for PC.

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Conflicts of interest
There are no conflicts of interest.

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